THE ELECTRON MICROSCOPY OF MYELINATED NERVE

by

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Nerve is one of the most important animal tissues now accessible to study with the electron microscope. This importance lies partly in the increased understanding of the mechanism of impulse propagation that can be expected to result from a knowledge of the macromolecular composition of nerve and partly from a need for this knowledge in the study of neurotropic viruses. Our studies of nerve have been carried out largely to meet this need.

The present paper describes observations made on the fine structure of myelinated nerve. Intact nerve fibers are generally too thick for examination and this has restricted investigators until recently to the study of their fragments or expressed contents[2, 7]. Sections thin enough for electron microscopy can, however, now be cut and the photographs of this paper have all been made from such sections. Electron micrographs of sectioned nerve have been published[4] but the sections shown there were obviously grossly damaged.

Sections of nerve thin enough for satisfactory electron microscopy have not been especially difficult to cut, but it has been hard to find a fixative that would not introduce obvious artifacts. We have not solved the problem of making electron microscopic sections devoid of artifact, but we have produced thin sections that reveal without serious distortion the same structural elements seen under the optical microscope together with much new detail within these elements. These results suggest that a more extensive study of methods of specimen preparation might lead to sections devoid of recognizable artifacts; they demonstrate that electron microscopy brings out much new information about the fine structure of nerve.

In these experiments the *nervus ischiadicus* of adult rabbits was used. Fragments of tissue, 1–2 mm on a side, were immersed in fixative as soon as excised. Fixatives as different as formalin, osmic acid, mercuric chloride, potassium bichromate and chromic acid were tried, and also various of their combinations corresponding to the more common histological formulas. In spite of the well-known inadequacy of formalin as a cytoplasmic fixative and the apparent advantages that often are gained by combining it with some of the other fixatives just mentioned, we have found that for the nerve examined here none of these other fixatives was as good as 4% formalin nor did the addition of any of them materially improve the ability of formalin to preserve all the nerve compo-

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nents known to be present. Osmic acid, which has in the past been favoured by histologists using the optical microscope for the study of nerve, introduced an especial amount of damage perceptible on the electron microscopic scale; from this standpoint it is the worst fixative we have tried. At the outset of this investigation alcohol was used as dehydrating agent but later pyridine was substituted when its superior action was discovered.

The freshly fixed pieces of nerve were washed, dehydrated, embedded in methacrylate and sectioned according to the expansion cutting method of NEUMAN, BORYSKO AND SWERDLOW[5]. After being floated off on dioxane-water, the suitably thin sections were picked up on a microscope slide and coated, after dissolving the methacrylate, with collodion, refloated on water, mounted on grids and shadowed with palladium to give adequate stability under the electron beam. In some cases the sections were placed as cut on a formvar coated grid and shadowed after dissolution of the methacrylate. Such preparations are difficult to make but the great clarity of their fine detail, unobscured by collodion, makes them sometimes well worthwhile. All observations were made with an RCA type EMU microscope at initial magnifications that were as low as 1,000 and as high as 12,000 ×.

The classical elements of the nerve fiber, *e.g.*, the axon, myelin sheath and neurilemma and the relation between the individual nerve fibers and the endo-, peri- and epineuria have been easy to recognize on the electron micrographs (Fig. 1). The single nerve fibers vary greatly in thickness; this is well brought out in cross sections (Fig. 2) photographed at low magnification. The smallest are about 2 micra in their diameter, the biggest 20–25 micra. The ratio between the thickness of the axon and the enveloping sheaths depends mainly on the thickness of the myelin sheath and this too varies greatly in different peripheral nerves. It is a familiar observation of optical microscopy that in some fixed preparations the axon has shrunken down to a thin axial thread or, in cross section, to a dark spot which gives the impression of a larger myelin space. We have sometimes observed this axonic shrinkage in electron microscopic preparations but believe that it can be recognized when it takes place. In the nerve investigated here the axon has from one-third to one-half the diameter of the whole nerve fiber. Sometimes the diameter of an axon seems somewhat variable, but this may be due to a shift in the plane of cutting arising from the fact that the fibers within a bundle are not strictly parallel. The neurilemma has a negligible thickness compared to myelin or the axon, being about 500 A across (Figs 1, 2, 15 and 16).

It has been difficult to decide if a special membrane separates the axon and the myelin because there is usually no detectable change in electron opacity in passing from one to the other. Pictures of good sections do indicate a continuous wall around the axon (the axolemma of MAUTHNER), but this seems to be formed by components of the myelin sheath rather than by a separate and distinct membrane interposed between the two. Sometimes this wall appears continuous with contents of the axon as well as with the myelin, but the recent suggestion[4] of an axolemma extending into the axon "where it forms the basis for the fine cobweb-like network bridging the lumen of the axis cylinder" is more sweeping than our photographs justify.

Under the microscope the axon is electronically less dense than the myelin sheath. According to ENGSTRÖM AND LÜTHY, there is no lipid in the axon[3]; its electronic transparency indicates that it has a low protein content. In many longitudinal sections the axon shows an undulating fibrillar structure parallel to the fiber axis (Fig. 1). This

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appears to be produced by thin fibrils about 200 A in diameter. These individual threads are most clearly seen in (Fig. 3) preparations from which the methacrylate has been removed. Sometimes they have carried adhering granules but no definite fine structure has ever been observed in them. In appearance they resemble the threads observed by SCHMITT[6] in pressed-out juice of the giant axon of the squid.

In certain other preparations the axon has appeared to have a rather different structure. As Figs 4, 5, 6 indicate the threads are not separate but are linked up to form an irregular three dimensional network. The present experiments do not demonstrate if both these axon structures should be considered as real.

Only one additional structural element has been seen in the axon. It consists of rod-shaped, very opaque particles of different lengths and widths (Figs 7, 1, 11). They are scattered here and there in axons always with their long axes in the direction of the fiber axis. Rod-shaped particles identified as mitochondria have been seen optically in the motor cells of the spinal cord and occasionally in the axons of peripheral nerves. They may be identical with the largest of these particles in electron micrographs.

The myelin sheath is structurally more complicated. As seen in our electron micrographs, it is a highly anastomosing lamellar net with a predominantly radial arrangement. This is indicated in both the longitudinal and cross sections (Figs 1, 2, 11). BEAR, PALMER AND SCHMITT[1] have concluded on the basis of polarized light and X-ray diffraction studies that the lipid-protein components of the sheath consist of layers wrapped concentrically about the axon. The amount of lipid remaining in our sections is unknown since at least part was removed during the treatment incident to specimen preparation. The same type of net is, however, always found in well-preserved nerve section and it probably should be considered as a residual framework of structural elements present in the complete sheath. In the electron micrographs roughly half the volume of the myelin sheath appears occupied by the lamellae while the rest is empty space. It is interesting in this connection to note that ENGSTRÖM AND LÜTHY[3], using their new characteristic X-ray absorption measurement of mass, have recently found 50% lipids in the middle part of the myelin sheath. In some well-preserved nerves the lamellar structure immediately surrounding the axon is finer than the rest and the voids correspondingly smaller (Figs 2, 10, 11, 12); in others the same texture is maintained throughout. The non-uniformity in structure of the myelin outwards from the axon is compatible with the finding that there is a non-uniform X-ray absorption in the sheath of lipid-extracted single fibers of the sciatic nerve of the frog. As was the case with the fine structure of the axon, evidence is not yet sufficient to show how much of the myelin fine structure, and the differences in this structure, are to be attributed to changes taking place during specimen preparation.

The *clefts* or *incisures of* SCHMIDT-LANTERMANN are a conspicuous feature of many electron micrographs. In poorly preserved nerves (Fig. 8) they appear as symmetrical rifts extending diagonally across the myelin sheath from the neurilemma to the axon which in such regions has usually been constricted and is especially dense. In better preserved nerves they are evident membranes or partitions that run symmetrically inwards to the axon and make a very acute angle with its axis. Such electron micrographs as Fig. 9, 10, 12 demonstrate that the incisures are membranes that can be considered as the surfaces of truncated cones based on the neurilemma and terminated at their intersections with the axon. They should therefore be designated as the *conic membranes* rather than the *clefts* of SCHMIDT-LANTERMANN. Although the membranes usually run

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in the same direction along a single fiber they are sometimes seen close together and opposite to give the impression of double funnels. The distribution of these membranes along a fiber is very variable but in our preparations they have been on the average 10–20 micra apart.

The finer texture of the inner portion of the myelin is especially evident where one of the conical membranes approaches the axon (Figs 10, 11, 12). In these regions the otherwise marked radial arrangement of the myelin elements is almost completely absent. Another view of the myelin structure is evident in sections that pass only through the outer part of the myelin sheath. Then as Fig. 13 indicates, the myelin has the aspect of a sheet perforated by roughly circular holes of various sizes.

The outermost sheath, the neurilemma, is seen in sections as a thin well-defined line ca 500A thick (Figs 1, 2, 9, 12, 13). Sections cut tangentially to the nerve fiber suggest that it is fibrous. Thus (Fig. 14) where the plane of cutting traverses the neurilemma, one sees it as a sheet which seemingly is a close net of very delicate fibers; no other membranous structure has been visible in our photographs nor have we seen the thin, flat, cytoplasmic network of SCHWANN's cells enveloping the nerve fiber. Even at the nodes of RANVIER where the neurilemma is especially noticeable (Figs 15, 16) it has appeared exclusively fibrous. The comparatively few cell nuclei (Fig. 2) seen in our preparations have not been connected with the neurilemma and consequently it has not been possible to determine if they belonged to Schwann's cells or to mesenchymal connective tissue cells.

Though we have examined lengths of nerve in many preparations, few nodes of RANVIER have been observed. Whenever a node was found others were seen nearby in practically all the adjacent fibers of a bundle. The nodes thus occur in groups at infrequent intervals along the nerve. What has been seen of them corresponds to the findings with the optical microscope. Unfortunately, the best nodes were encountered while trying out a fixative containing mercuric chloride which gave relatively poor preservation of the myelin. This damage is obvious at the nodes of Figs 15 and 16. Here the neurilemma dips inward to a constricted axon. This constriction extends a short distance on either side of the point where the neurilemma reaches the axon and in this region the internal "threads" seem especially well oriented and closely packed ("spiny bracelet of NAGEOTTE"). The axon continues uninterrupted across a node with no hint of a material crossplate corresponding to the bright line visible under the optical microscope.

The numerous individual fibers of the peri-, epi- and endoneurium form a single system. The perineurium consists of large bundles of the fibrils in dense, well-defined layers (Figs 17, 18). The fibrils of the epi- and endoneurium are not so well arranged but are found going here and there between the nerve fibers (Fig. 19). The fine structure of these fibrils can best be studied in shadowed preparations mounted on formvar-coated grids. Some of them then appear as continuously cross-striated with a periodicity of about 200A while others show the ca 640A repetitions characteristic of collagen (Fig. 20). They are very uniform in diameter though those in contact with nerve fibers are thinner than the rest. Evidently they are structurally like the connective tissue found associated with other bodily structures.

CONCLUSIONS

The electron micrographs described in the preceding paragraphs provide a first step towards an understanding of the macromolecular structure of nerve. They show

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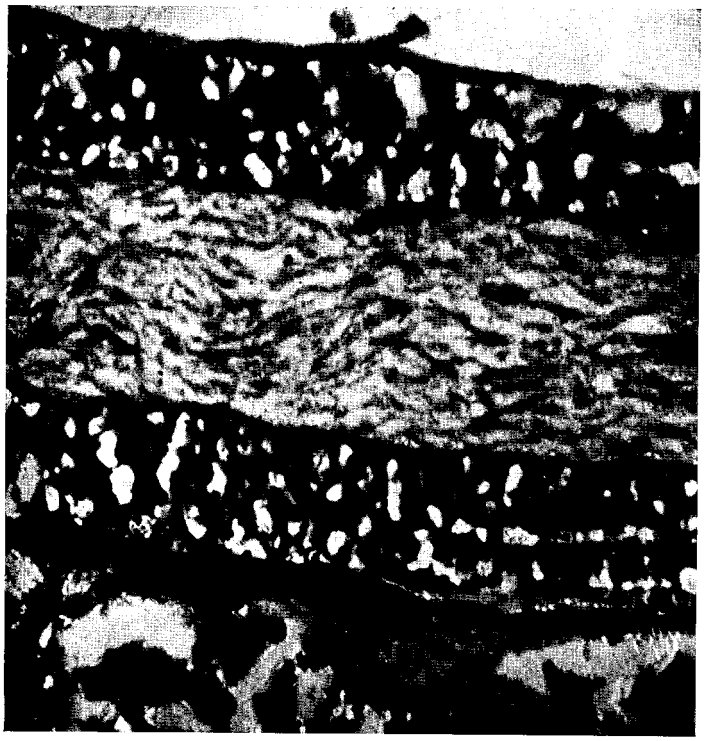

Fig. 1. A longitudinal section approximately through the center of a single nerve fiber. The light central axon shows the presence of many thin wavy "threads" and a few thicker objects that may be aggregates of these threads. The thickest objects may be examples of the "mitochondria" sometimes seen under the optical microscope. Outside the axon are the denser walls of the compartmented myelin which in this fiber has about two-thirds the diameter of the axon. A thin neurilemma enveloping the fiber is best seen at the top.
Magnification = 5400 ×

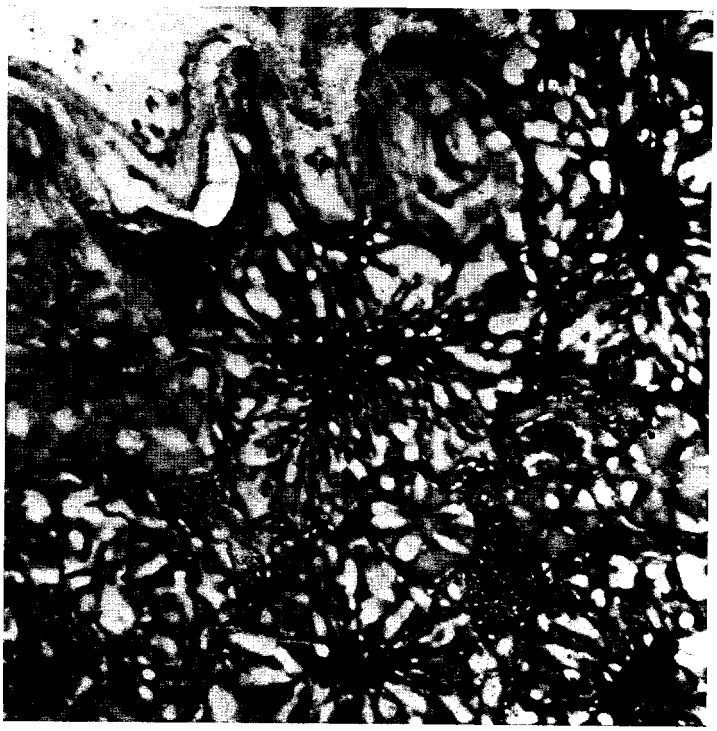

Fig. 2. A transverse section through part of a nerve showing fibers of various sizes. The perineurium, surrounding the entire nerve, appears at the upper left of this picture.
Magnification = 4900 ×

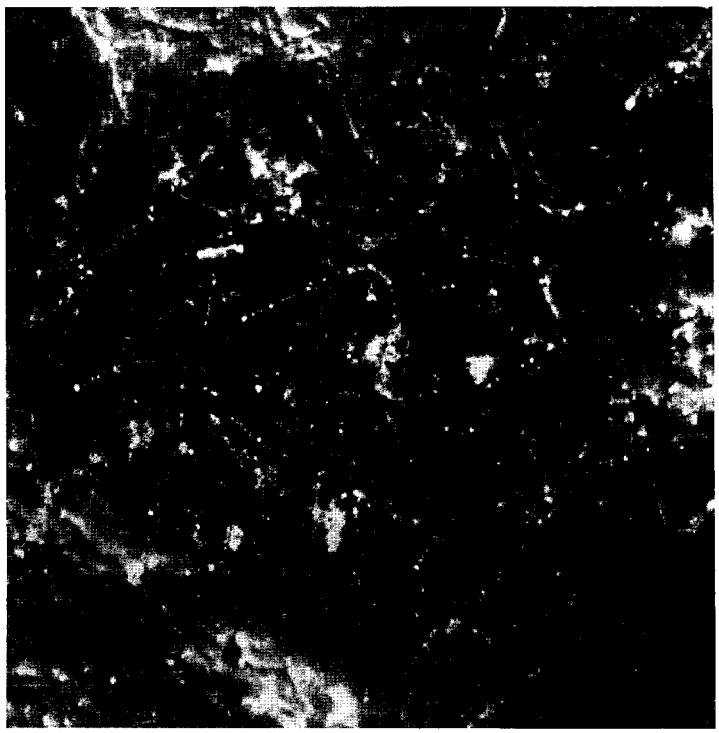

Fig. 3. Axon filaments seen at a relatively high magnification. They all have about the same diameter. To show these filaments the embedded section was mounted on formvar before removal of the methacrylate. Unlike the two preceeding electron micrographs this is a negative print to bring out the effect of the shadowing. Magnification = 27000 ×

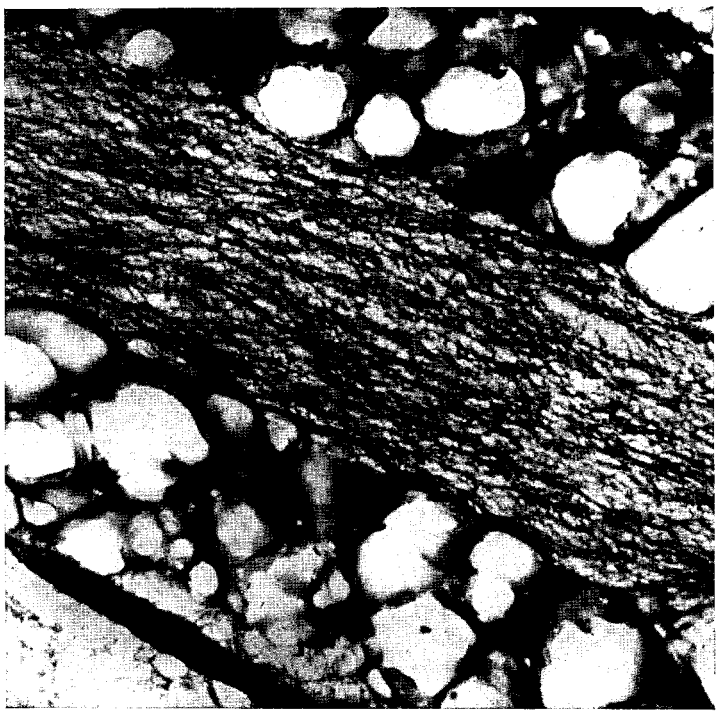

Fig. 4. A longitudinal section of a fiber at a higher magnification than Fig. 1. The filaments of the axon here seem to be tied together to form a three-dimensional mesh. The neurilemma is visible in the lower left corner. A positive print.
Magnification = 12000 ×

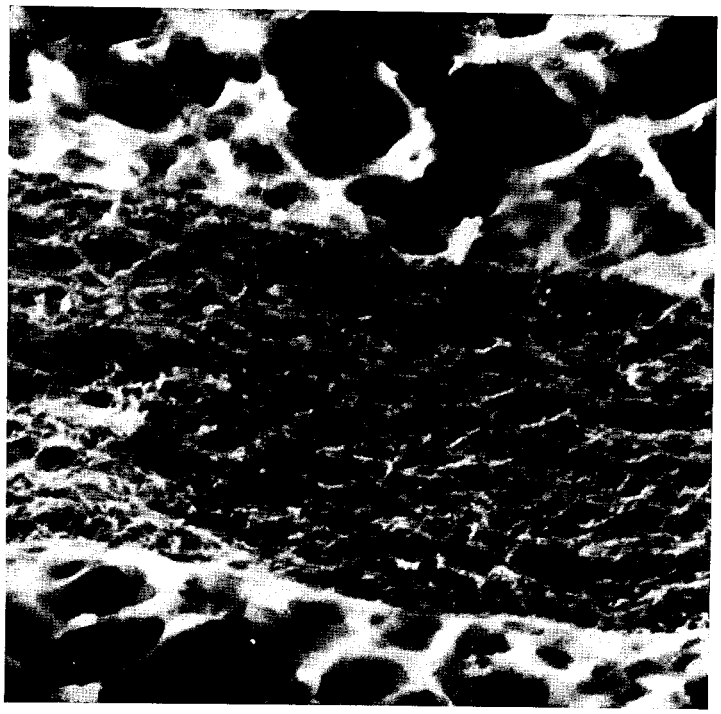

Fig. 5. A similar longitudinal section printed as a negative to emphasize the three-dimensional character of the axon mesh as revealed by the shadowing. Magnification = 19000 ×

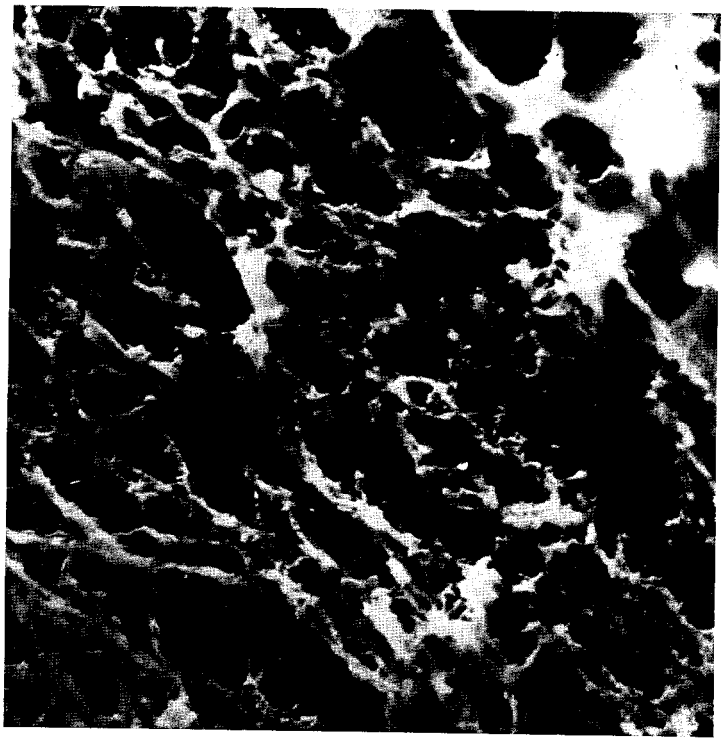

Fig. 6. A portion of the axon in another section at a still higher magnification. Negative print. A bit of myelin appears at the upper right. The absence of a separate axolemma is evident in each of the three last photographs. Magnification = 17000 ×

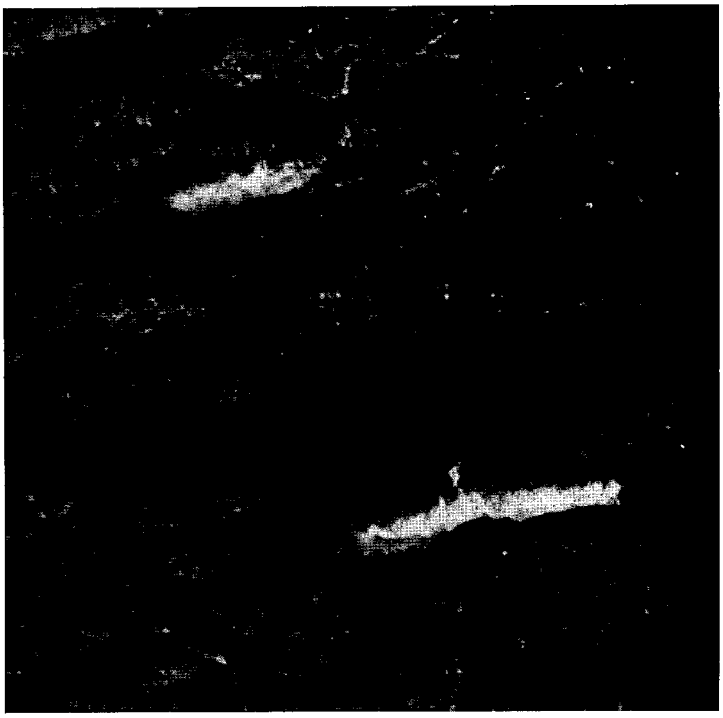

Fig. 7. A portion of axon in a longitudinal section showing two "mitochondria". The filaments are visible though less clearly than in some of the preceeding photographs because they are partly obscured in a thin film of supporting collodion added after removal of the methacrylate.
Magnification = 18000 ×

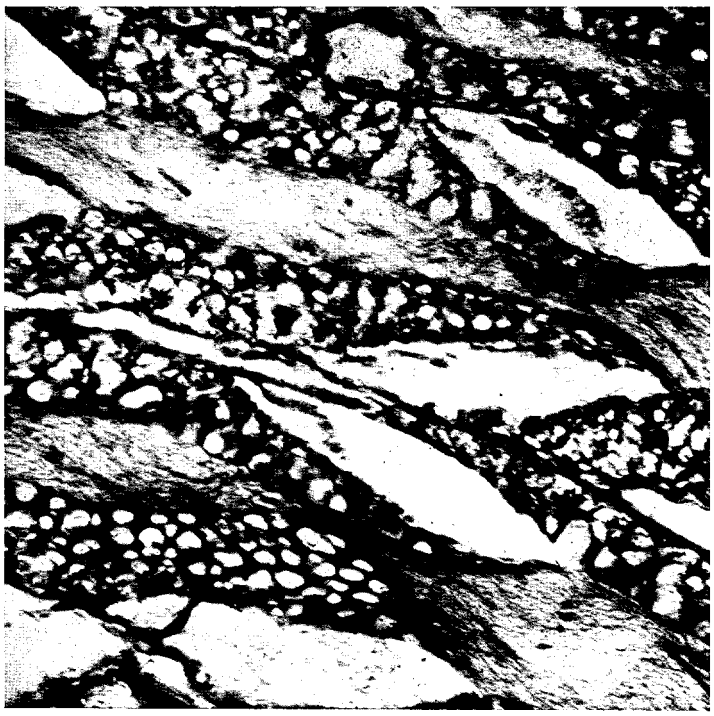

Fig. 8. Two fibers of a nerve showing clefts of Schmidt-Lantermann. A second cleft in the upper fiber is partly visible at the left. The compression of the axon, especially severe in the lower fiber, indicates that the break forming the "cleft" occurred before dehydration.
Magnification = 4500 ×

Fig. 9. Undamaged "cleft" showing that it is really a conical membrane traversing the myelin. Neurilemma appears at the lower right.
Magnification = 8000 ×

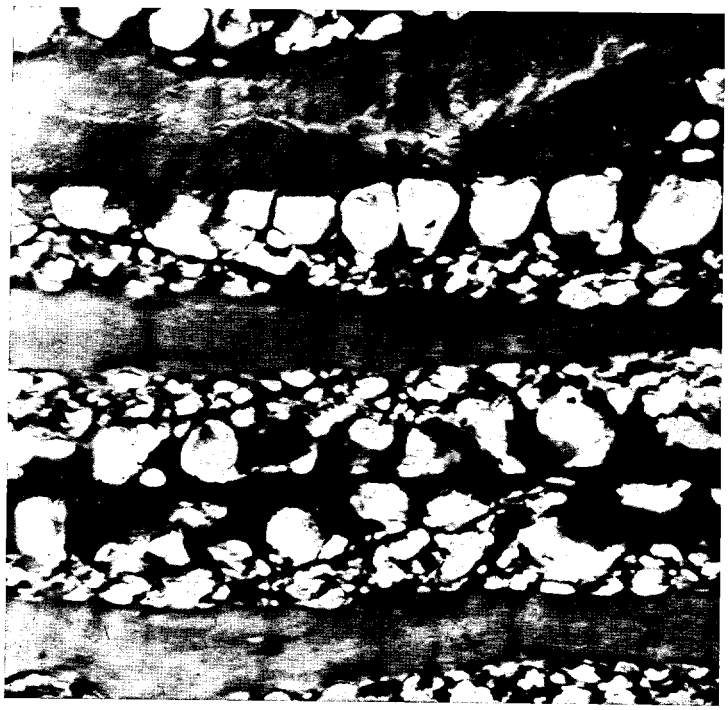

Fig. 10. The two fibers at the bottom of the picture contain unbroken "clefts" running in opposite directions. The finer texture of the myelin around the axon near these "clefts" is apparent.
Magnification = 4300 ×

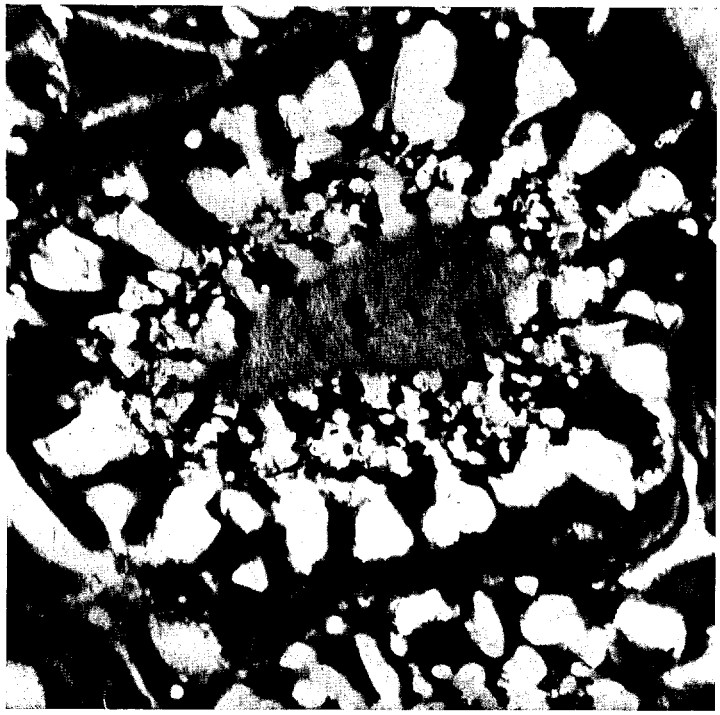

Fig. 11. A transverse section through a fiber revealing the finer texture of the myelin immediately surrounding the axon. Note the "mitochondria" within the axon. Magnification = 5000 ×

Fig. 12. Another, almost transverse, section through several fibers. The finer myelin around the axon is conspicuous in the large central fiber. Magnification = 4500 ×

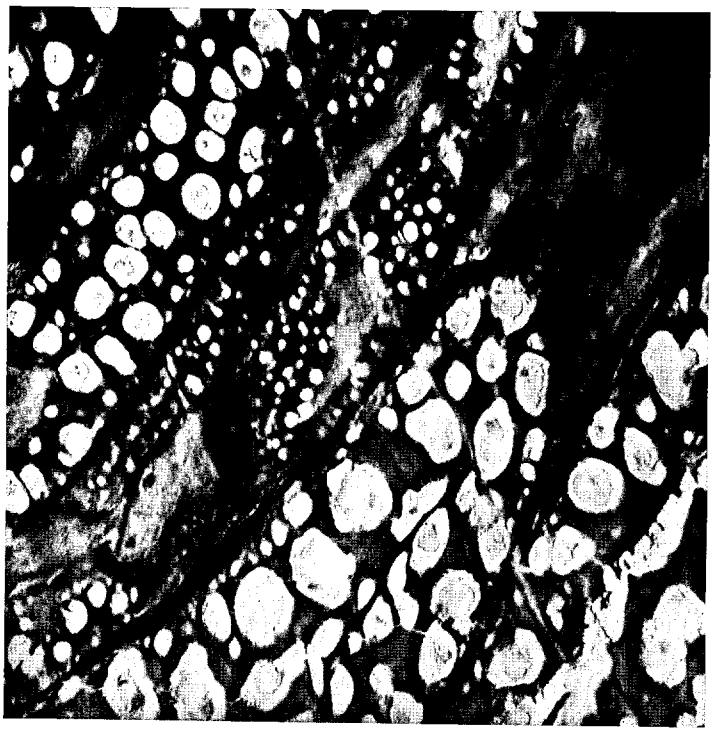

Fig. 13. Tangential, nearly longitudinal, section cutting only the myelin of several fibers. Note the difference in mean size of the holes in adjacent fibers. Magnification = 4300 ×

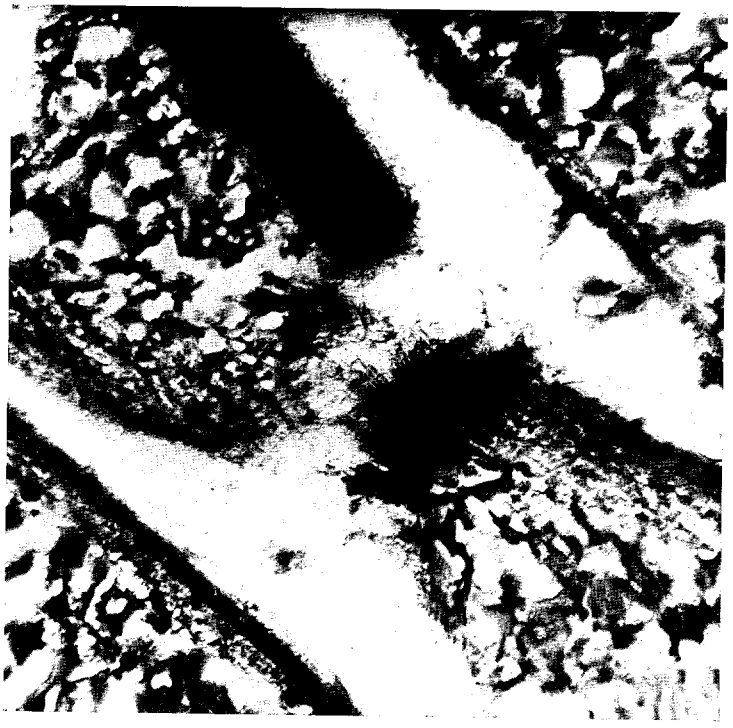

Fig. 14. A nearly longitudinal section through a fiber presumably at a node but at a level that does not involve the axon. The dark material at the end of the right hand piece and along the top of the left piece of fiber shows clearly the fibrous character of the neurilemma. Magnification = 4500 ×

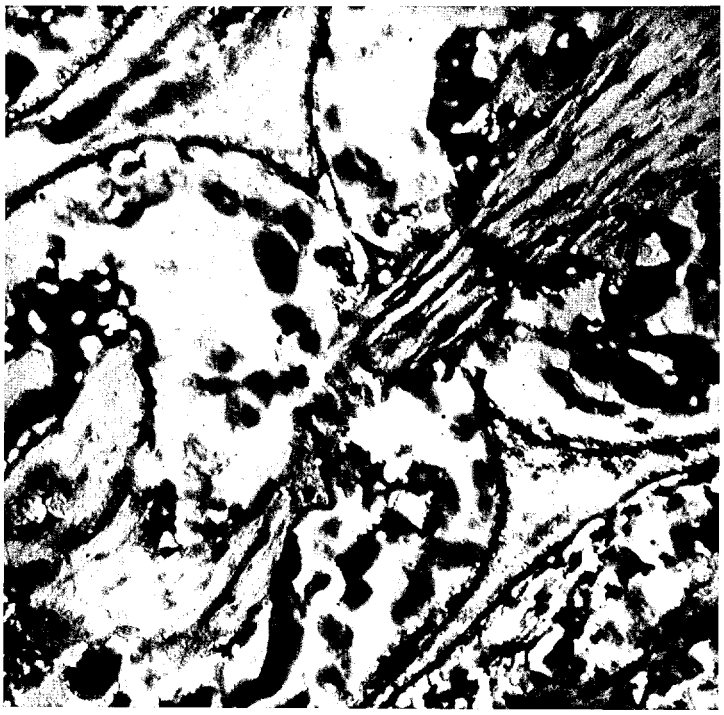

Fig. 15. A longitudinal section through a node of Ranvier at a level which includes some axon. Damage to the myelin around the node is clear. Magnification = 4500 ×

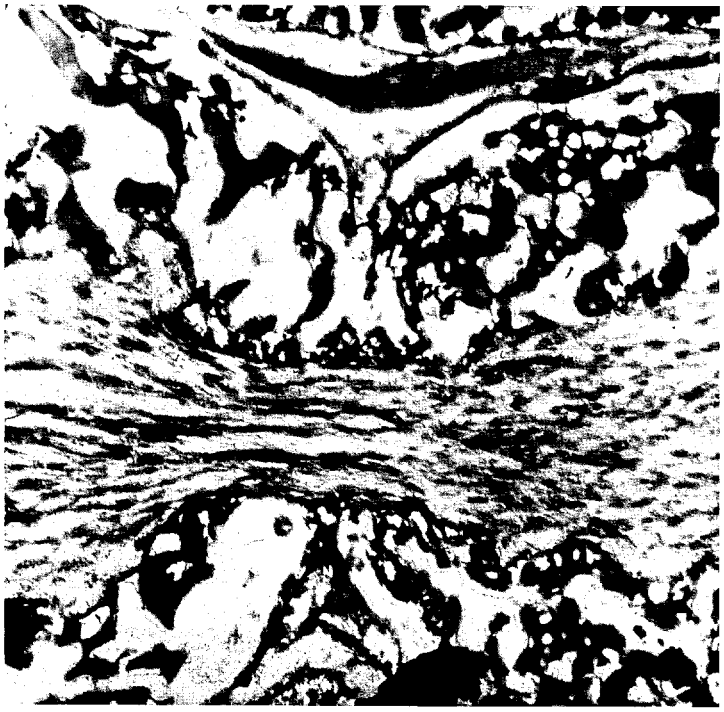

Fig. 16. A longitudinal section through a node almost midway along the axon. The continuity of the axon across the node is evident. Magnification = 4500 ×

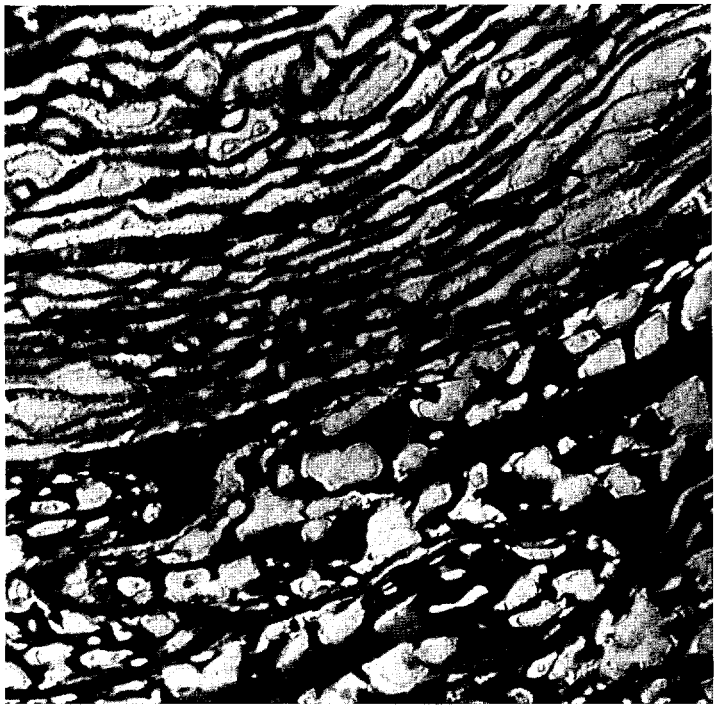

Fig. 17. A section through the outer part of a nerve bundle showing at the bottom parts of fibers, at the top successive layers of the fibrous perineurium. Magnification = 4500 ×

Fig. 18. A part of the perineurium at a higher magnification showing at the top some of the individual fibres of which it is composed.
Magnification = 6000 ×

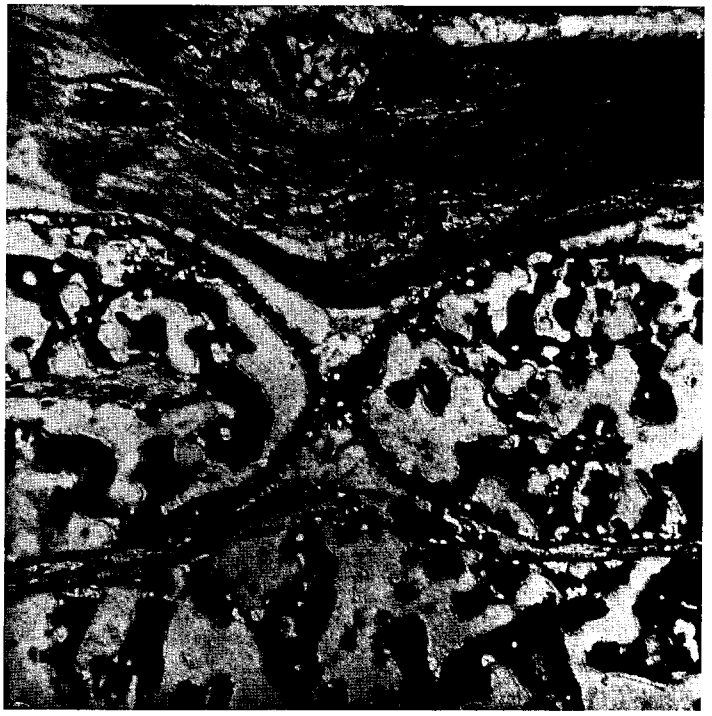

Fig. 19. A bundle of filaments of epineurium dipping to fill in the space around a node. Magnification = 4500 ×

Fig. 20. Filaments of perineurium at a higher magnification to show its characteristic cross striations of connective tissue. A negative print to bring out the three dimensional effect of the shadowing. Magnification = 28000 ×

that the *nervus ischiadicus* of the adult rabbit is a bundle of separate fibers of various diameters embedded in and bound together by bundles of connective tissue filaments which have the same characteristic cross striations as collagen from other sources. With the best fixatives used here the single nerve fiber appears composed of a very thin outer neurilemma covering a myelinated region and a central axon each of which has about half the diameter of the fiber. The myelin, from which lipid has undoubtedly been removed by the methods of specimen preparation, appears as an anastomosing lamellar net or cage which is often of a finer texture in the region surrounding the axon. In a fiber it is interrupted at intervals by conical partitions or membranes that correspond to what optical microscopy has designated as the clefts of SCHMIDT-LANTERMANN. The myelin net terminates at the axon in what appears to be a continuous barrier which is, however, no thicker than the partitions of the net. The filamentous material within the axon sometimes has appeared as threads running lengthwise of the fiber and sometimes as such threads bound together by cross filaments to form a delicate network. The axon is constricted but not interrupted at the nodes of RANVIER.

It is not yet possible to know if certain of the fine details seen in these electron micrographs are artifacts produced during specimen preparation. This can only be learned after extensive studies with a variety of fixatives and dehydrating agents. Nevertheless, the present results do demonstrate under the electron microscope the principal features of nerve structure previously seen with the optical microscope, they prove that the methods of electron microscopy can now extend our knowledge of the fine structure of nerve tissue, and they show the general direction which should be taken in work designed to further this knowledge.

SUMMARY

Electron micrographs have been made of sections through the myelinated *nervus ischiadicus* of the adult rabbit. For this purpose excised nerves have been preserved in various fixatives and dehydrated in alcohol or pyridine. These photographs demonstrate various aspects of the macromolecular texture of the axon and its contents, of the myelin and of the fibrous sheets in which the individual nerve fibers are embedded to make a bundle.

RÉSUMÉ

Nous avons pris des electromicrographies de sections à travers le *nervus ischiadicus*, entouré de sa gaîne de myéline, du lapin adulte. A cet effet les nerfs ont été préservés dans divers fixateurs et déhydratés à l'alcool ou à la pyridine. Ces photographies montrent les divers aspects de la texture macromoléculaire du cordon nerveux et de son contenu, de la myéline et des couches fibreuses qui réunissent en faisceaux les fibres nerveuses individuelles.

ZUSAMMENFASSUNG

Elektronenmikrographien von Schnitten durch den, von seiner Myelinscheide umgebenen *Nervus ischiadicus* vom erwachsenen Kaninchen wurden aufgenommen. Zu diesem Zwecke wurden die Nerven in verschiedenen Fixiermitteln konserviert und mit Alkohol oder Pyridin dehydriert. Diese Photographien zeigen verschiedene Aspekte des makromolekularen Aufbaus des Nervenstranges und seines Inhaltes, des Myelins und der fasrigen Schichten welche die einzelnen Nervenfasern zu einem Bündel vereinigen.

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Received May 13th, 1950